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Effect of 15% Carbamide Peroxide on the Surface Roughness and Adhesion of *Streptococcus mutans* to Microhybrid Composite Resin and Giomer

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ABSTRACT

Introduction: Adhesion of bacteria, especially *Streptococcus mutans* (*S. mutans*), to the surface of tooth restorations is a factor in the etiology of secondary caries. Given the ever-increasing popularity of bleaching procedures, the aim of the present study was to evaluate adhesion of *S. mutans* and surface roughness (SR) of microhybrid composite resin and giomer subsequent to the application of 15% carbamide peroxide.

Materials and methods: Twenty disk-shaped samples were prepared from each material, measuring 8 mm in diameter and 2 mm in thickness. Then, the samples of each material were divided into two groups (n = 10): (a) microhybrid without bleaching; (b) microhybrid with bleaching; and (c) giomer without bleaching; and (d) giomer with bleaching. The samples in groups I and III were immersed in artificial saliva for 14 days without any bleaching procedure; the samples in groups II and IV underwent a bleaching procedure on their polished surfaces with 15% carbamide peroxide for 14 days (4 hours of bleaching and 20 hours of immersion in artificial saliva). The SR of all the samples was determined with the use of a profilometer. The samples were added to the culture medium after 4 hours of placement in a microbial suspension at 37°C; after 24 hours of incubation at 37°C, the bacterial counts, indicating the number of bacteria adhering to the surface, were determined by counting them in the plates containing the solid culture medium.

Results: The type of the restorative material had a significant effect on SR, with greater SR in giomer (p = 0.03). However, bleaching had no significant effect on SR (p = 0.099). In relation to the rate of bacterial adhesion (BA), both the types of the restorative materials and bleaching procedures were significantly effective; in this context, there was more BA in microhybrid composite resin samples that did not undergo bleaching (p < 0.001). Bleaching resulted in the adhesion of *S. mutans* to the surface of both materials decrease. Pearson's correlation coefficient did not reveal any correlation between BA and SR (p = 0.42).

Conclusion: The BA was higher in microhybrid composite resin, and SR was higher in giomer. The BA was higher in samples that did not undergo a bleaching procedure.

Clinical significance: There is no change in the SR of microhybrid composite resin and giomer after application of 15% carbamide peroxide; therefore, it is not necessary to polish or replace these restorative materials after bleaching. In addition, use of 15% carbamide peroxide does not increase caries risk.

Keywords: Carbamide peroxide, Giomer, Microhybrid composite resin, *Streptococcus mutans*, Surface roughness.

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INTRODUCTION

Discolored vital teeth are treated in different ways to improve their esthetic appearance, including the use of crowns, direct and indirect veneers, composite resin restorations, and bleaching, which is the most conservative technique.¹ At-home bleaching is the most commonly recommended treatment modality for vital teeth.² However, these teeth might have tooth-colored restorations. The clinical longevity of tooth-colored restorations might be affected by chemical processes of bleaching agents.³ The effects of bleaching agents include changes in surface morphology and in the physical and chemical properties of tooth-colored restorative materials.³ In addition, it has been shown that the SR of composite resins is affected to a great extent by bleaching procedures.¹ It appears bleaching agents increase the adhesion of cariogenic bacteria to the external surfaces of dental materials.⁴ Adhesion of bacteria to the surfaces of composite resins and other restorative materials is a factor in the etiology of recurrent caries.⁵ Adhesion of *S. mutans* has the strongest correlation with caries experience.⁶

Composite resins are direct restorative materials that meet, in the best way, the requirements of the preservation of tooth structure, high esthetic appearance, and

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longevity.⁷ At present, microhybrids are the most commonly used direct restorative materials used, and in all the cases, in which a composite resin with high polishability and good mechanical properties is required, microhybrid composite resins are recommended; in fact, microhybrid composite resins are considered all-purpose universal composite resins.^{7,8} Based on the results of recent clinical studies, giomer, too, is a suitable restorative material for carious teeth in areas in which esthetic appearance is very important.⁹ The giomer technology relies on prepared glass-ionomer, which is placed as an additional phase within composite resin.^{10,11} In patients with poor oral hygiene and a cariogenic diet, giomer might be a good choice for decreasing the effects of a poor oral environment due to its capacity to release fluoride and high polishability.^{12,13} Recently, controversies have risen over the effect of bleaching agents on the physical and chemical properties of restorative materials. Two studies have shown that highly polished composite resin restorations possibly exhibit less bacterial accumulation. In contrast, Yamuchi et al¹⁴ showed that *S. mutans* exhibits greater adhesion to smooth surfaces, while Yamamoto et al¹⁵ reported no relationship between SR of composite resins and BA. Mor et al⁴ too showed that *S. mutans* has a higher capacity to adhere to bleached composite resin surfaces. Kimyai et al¹⁶ showed that adhesion of *S. mutans* to giomer is less than that to composite resin. Some studies have shown that the adhesion of *S. mutans* is higher to bleached enamel surface.¹⁷⁻¹⁹ However, in relation to the SR of bleached enamel, both an increase¹⁷ and absence of any change¹⁸ have been reported. In relation to the effect of bleaching on SR of restorative materials, too, contradictory results have been reported. Some studies have shown that SR of microhybrid composite resins does not noticeably change after bleaching.^{20,21} In addition, some studies have shown that SR of restorative materials increases after bleaching with carbamide peroxide.²²⁻²⁶ Mohammadi et al,²⁷ too, showed a statistically significant difference in the SR of giomer before and after bleaching with carbamide peroxide. Since no studies are available on the adhesion of *S. mutans* to microhybrid composite resins, as they are the most commonly used tooth-colored restorative material, and giomer, which has antibacterial effects, after bleaching procedures, and considering the discrepancies in relation to the SR of these materials after bleaching, the present study was designed to evaluate the effect of 15% carbamide peroxide and the material type on the adhesion of *S. mutans* and SR of these materials. The null hypotheses tested were as follows: (1) There is no difference in the SR of the study groups. (2) There is no difference in the SR of the study groups before and after bleaching. (3) The type of the restorative material and bleaching agent have no effect on the

adhesion of *S. mutans*. (4) There is no correlation between the adhesion of *S. mutans* and SR of the materials.

MATERIALS AND METHODS

The protocol of the study was approved by the Ethics Committee of Tabriz University of Medical Sciences under the code IR.TBZMED.REC.1395.526. Based on the results of a pilot study and by considering a difference of 204 units in the BA variable between the two groups, with and without bleaching with standard deviations of 150.68 and 122.76 respectively, and by considering $\alpha = 0.05$ and a study power of 80%, the sample size was estimated at $n = 8$ in each group; however, to increase the validity of the study, 10 samples were included in each group. Therefore, a total of 40 samples (10 in each group) were evaluated in this study. Four extra samples (one from each group) were included as controls.

A total of 20 disk-shaped samples were prepared within cylindrical plastic molds from microhybrid composite resin (Filtek Z250™, 3M ESPE, St. Paul, Minnesota, USA) and 20 samples from giomer (Beautifil II, Shofu Dental Corporation, Osaka, Japan), measuring 8 mm in diameter and 2 mm in thickness. First, a transparent matrix band was placed on a glass slab under the mold. Composite resin was placed in 2-mm-thick layers on it, and then, a transparent matrix band was placed on it. A glass slab was placed tightly on it to achieve a smooth surface.

The samples were light-cured with a halogen light-curing unit (Elipar 2500, 3M ESE) at a light intensity of 480 to 520 W/cm². A radiometer (Optilux, Model 100SDS, Kerr, Danbury Connecticut, USA) was used to evaluate and make sure of the intensity of curing light. The tip of the light-conducting device was placed in contact with the glass slab, and light-curing was carried out for 40 seconds from both sides. One surface of the samples was polished with Solfex disks in a low-speed handpiece in one direction in three states of moderate, fine, and superfine.³ The final thickness of the samples was checked with the use of a micrometer (Ultra Cal Mark 3 Flower Tools and Ins., Sylvac Newtown Mt, USA). After polishing, the samples were subjected to an ultrasound cleaning procedure with distilled water for 2 minutes and then immersed in 37°C distilled water for 24 hours.³

The samples of both materials were randomly assigned into two groups ($n = 10$) as follows:

- *Group I*: Microhybrid composite resin samples without bleaching
- *Group II*: Microhybrid composite resin samples after a home bleaching procedure with 15% carbamide peroxide
- *Group III*: Giomer samples without bleaching
- *Group IV*: Giomer samples after home bleaching with 15% carbamide peroxide.

The samples in groups I and III were immersed in artificial saliva without any bleaching procedures. The samples in groups II and IV underwent a bleaching procedure with 15% carbamide peroxide on their polished surfaces for 14 days, consisting of 4 hours of bleaching every day, followed by 20 hours of storage in artificial saliva.

The artificial saliva was refreshed every day. The chemical composition of artificial saliva consisted of 0.04 mg of sodium chloride, 0.4 mg of KCl, 0.795 gm of $\text{CaCl}_2 \cdot \text{H}_2\text{O}$, and 0.69 gm of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 0.055 gm of $\text{Na}_2\text{S}_9\text{H}_2\text{O}$, dissolved in 1000 mL of distilled water at pH = 7.³

The bleaching agent (Opalescence PF 15% Carbamide Peroxide, Ultradent Products Inc., South Jordan, USA) was applied to the polished surface of each sample at a thickness of 1 mm for 4 hours each day; then the samples were immersed in artificial saliva at 37°C for 20 hours until the next round of the bleaching procedure. After 14 days, all the samples were immersed in distilled water at 37°C for 24 hours.³ At the end of the bleaching period, the SR of all the samples was determined with the use of a profilometer.⁹ In this test, the diamond rod of the instrument that measured 2 µm in diameter scanned the surface at three points at a constant rate of 0.1 mm/s using a force of 0.7 mN and the mean SR was reported using a numeric value (Ra). In groups I and III, after 14 days of immersion in artificial saliva, the samples were immersed in distilled water at 37°C. Then, the SR was determined with the use of a profilometer. Finally, the SR of all the four groups was compared.

Evaluation of Adhesion of *S. mutans*

Standard *S. mutans* bacterial species (ATCC25175), lyophilized according to the adjunctive protocol of the manufacturer (Biotechnology Research Center of Iran Scientific and Industrial Research Center), was activated in the tryptic soy broth (TSB) general culture medium and transferred onto the surface of TSB plates. Finally, a microbial suspension was prepared in 0.9% standard physiological serum from the colonies grown on TSB plates, at a concentration of 5×10^8 colony-forming units/mL using the plate counting technique. After preparation of the test samples, each sample was subjected to the microbial suspension within sterile Falcon tubes at 37°C for 4 hours. After incubation, the test materials were retrieved from the Falcon tubes containing the microbial suspension, under sterile conditions and rinsed with sterile physiologic serum three times to remove nonadhering microbial cells. The samples were transferred into new sterile Falcon tubes containing 0.9 standard physiologic serum and placed in a sonicator for 6 minutes to separate bacterial cells adhering to the surface to achieve a suspension. In the next stage, the test samples were retrieved and

added to the culture medium suspension. After 2 hours of suspension at 37°C, the bacterial counts, indicating the number of bacteria adhering to the surface, were determined comparatively in the study groups by counting them in the plates containing solid culture medium. All the tests were carried out in the presence of a positive control group (a culture medium containing bacteria with no samples) and a negative control group (a culture medium without bacteria but with samples), and each test was repeated 6 times.¹⁶ Then, adhesion of *S. mutans* was compared between the study groups.

Data on SR and *S. mutans* adhesion were analyzed with descriptive statistics (means and standard deviations) and two-way analysis of variance (ANOVA). Statistical significance was defined at $p < 0.05$. Pearson's correlation coefficient was used to evaluate the correlation between SR and *S. mutans* adhesion. Kolmogorov-Smirnov test was used to evaluate normal distribution of data. Independent samples t-test was used to evaluate the effect of bleaching on each material separately considering the normal distribution of data.

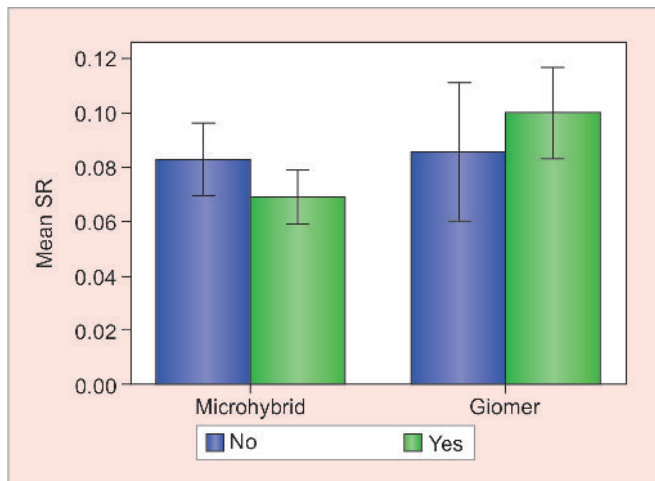
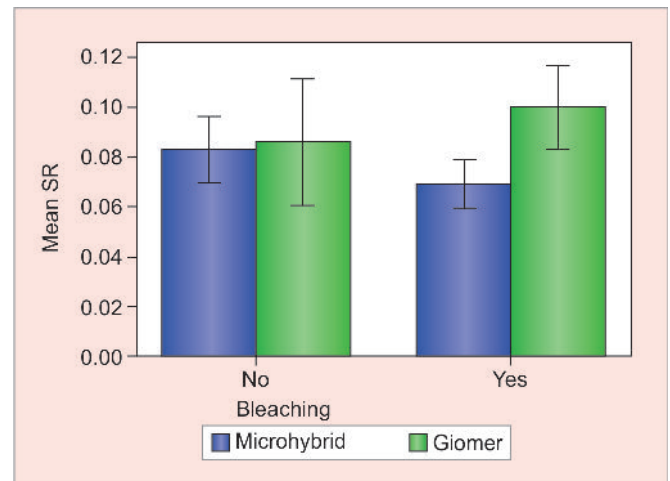
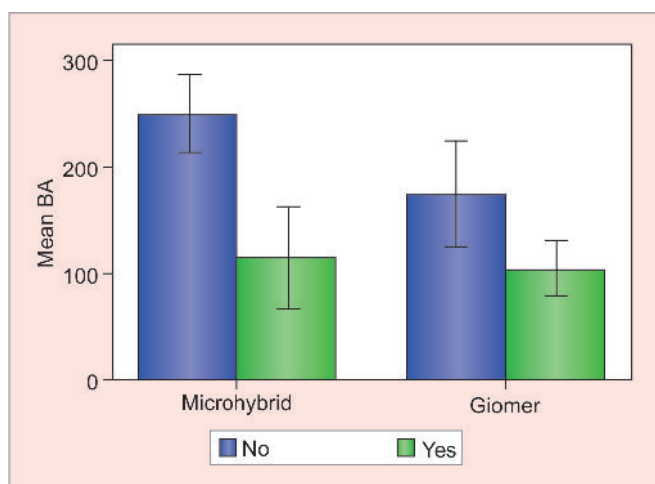
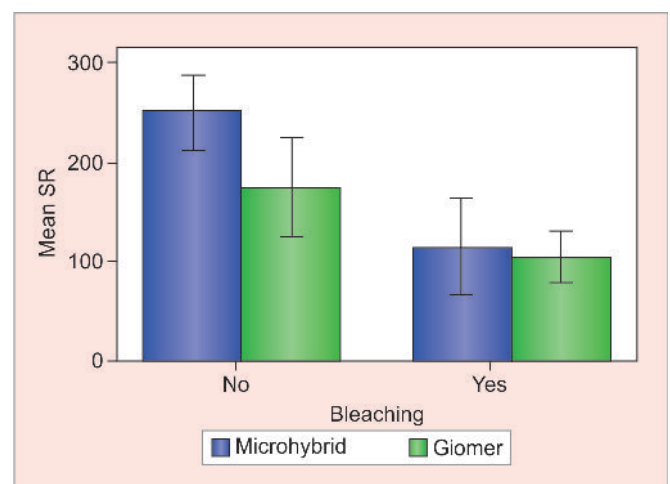
RESULTS

Table 1 presents the descriptive data on SR and BA in the study groups in terms of the restorative material and bleaching procedure. Two-way ANOVA was used to evaluate SR and BA with and without bleaching with the use of microhybrid composite resin and giomer, based on normal distribution of data as shown by Kolmogorov-Smirnov test at $p < 0.05$.

- Based on the analyses, of all the factors evaluated (the restorative material and bleaching), only the type of the restorative material affected SR significantly ($p = 0.03$), which was higher in giomer. However, bleaching had no significant effect on SR ($p = 0.68$; Graphs 1 and 2).
- In addition, based on the analyses, of all the factors evaluated (restorative material and bleaching), both the types of the restorative materials ($p = 0.03$) and bleaching ($p < 0.001$) affected BA significantly (Graphs 3 and 4).
- There was a 135-unit difference in BA in microhybrid composite resin with and without bleaching, with more BA in the group without bleaching. Based on the results of independent-samples t-test, the difference was significant ($p = 0.00$) (Table 1, Graphs 3 and 4).
- There was a 70-unit difference in BA in giomer with and without bleaching, with higher BA in the group without bleaching. Based on the results of independent-samples t-test, the difference was significant ($p = 0.01$; Table 1, Graphs 3 and 4).
- Pearson's correlation coefficient did not reveal any correlation between BA and SR ($p = 0.42$).

Table 1: Descriptive data on SR and BA in the study groups

Groups	Bleaching	n	Mean	Minimum	Maximum	Mean
Microhybrid						
SR	No	10	0.08 ± 0.01	0.05	0.10	0.08
	Yes	10	0.06 ± 0.01	0.05	0.10	0.07
BA	No	10	250 ± 52.54	175	365	232.50
	Yes	10	115 ± 67.61	30	225	90
Giommer						
SR	No	10	0.08 ± 0.03	0.01	0.12	0.10
	Yes	10	0.10 ± 0.02	0.05	0.12	0.10
BA	No	10	175 ± 70.47	65	270	165
	Yes	10	105 ± 36.59	40	150	105
Total						
SR	No	20	0.08 ± 0.02	0.01	0.12	0.1
	Yes	20	0.08 ± 0.02	0.05	0.12	0.07
BA	No	20	212.5 ± 71.69	65	265	227.5
	Yes	20	110 ± 53.16	30	225	100

**Graph 1:** Mean SR based on the restorative materials of this study**Graph 2:** Mean SR of the restorative materials in this study based on bleaching procedure**Graph 3:** Mean BA based on the restorative materials of this study**Graph 4:** Mean BA based on bleaching procedure in restorative materials of this study

DISCUSSION

The prognosis and longevity of restorations depend on the physical, biologic, and mechanical properties of restorative materials. The SR is one of the properties affecting the esthetic appearance, hygiene, plaque retention, and health of the gingival tissue adjacent to composite resin restorations. An increase in SR results in an increase in accumulation of food debris, formation of biofilm, and finally induction of periodontal diseases.²⁸ Adhesion of the bacteria to the surface of composite resin and other restorative materials is a factor involved in the etiology of recurrent caries.⁴ Adhesion of bacteria to rough surfaces in the oral cavity is mediated by different mechanisms, involving the type of the bacterial species and the surface itself.²⁹

The results of the present study refuted the first hypothesis of the study and showed that the type of the restorative material significantly affected the surface hardness, and giomer samples exhibited more SR. In the present study, a profilometer was used to determine SR, which is an accurate and appropriate technique. In the majority of studies, this instrument has been used to determine SR.^{30,31} Based on a report by Chung, when the SR is $<1\text{ }\mu\text{m}$, the surface is smooth visually. The size of the fillers is one of the factors that determines the SR and polishability of restorative materials.³⁰ A large size of the particles in composite resins might increase porosities in its structure. Polishing of composite resins is determined based on the longest diameter of fillers.³⁰ Composite resins with larger particles tend to exhibit more roughness when they are exposed to abrasive agents and abrasion resulting from foods and drinks.⁷

The two materials evaluated in the present study consisted of Filtek Z250 microhybrid composite resin and Beautifil II giomer. Filtek Z250 microhybrid composite resin has a matrix resin of bisphenol A (Bis) glycol dimethacrylate (GMA), Bis-ethoxylated dimethacrylate, and urethane dimethacrylate, with a filler size of 0.1 to $3.5\text{ }\mu\text{m}$. Its filler content is up to 60% of its volume. Beautifil II is a giomer composite with a matrix of Bis-GMA, triethylene glycol dimethacrylate, with a filler size of 10 to $20\text{ }\mu\text{m}$ and particles larger than $4\text{ }\mu\text{m}$. Its filler content is up to 68.6% of its volume. The filler sizes of both materials show that both materials have high polishability and preserve their polish for a long time. However, giomer has fillers with a matrix of glass-ionomer, and this matrix has a large amount of fluoride and metallic ions, and water can easily penetrate into it.¹⁶ Sorption of water results in the loss of particles a decrease in surface integrity, and a decrease in microhardness.³¹ A higher SR of giomer might be attributed to greater hydrolytic changes in it, resulting in the softening of its matrix.

In relation to the effect of bleaching on SR, it was shown that microhybrid composite resins that did not undergo bleaching, and giomer samples that were bleached exhibited greater SR; however, the differences were not significant. There is controversy in the previous studies over the effect of bleaching on SR of dental materials. Some studies have reported no changes in SR of the restorative materials after bleaching.^{20,21,23,31-33} Some others have reported decreases^{14,16} and some increases^{22-27,34} in SR.

Basting attributed the discrepancies in the results of SR of composite resins after bleaching to the chemical composition of composite resins and the combination of techniques and materials used for bleaching procedures.³⁵

In general, factors that have been reported by previous studies as reasons for increases in SR can be summarized as follows. The effect of free radicals on filler matrix interface and debonding of filler (loss of adhesion between the organic and inorganic matrix) result in the formation of microscopic cracks on the surface that can result in SR.^{25,27,30} Effect of different bleaching gels depends on the oxidation process which occurs in the organic matrix, facilitating sorption of water and resulting in the loss of particles, in a decrease in surface integrity, and in an increase in microhardness.³⁰ Hydrogen peroxide attacks the matrix and results in the softening of materials, leading to the loss of glass. In addition, light, too, can increase the effect of hydrogen peroxide on increasing SR.³¹ Munteanu, too, attributed the increase in SR to oxidation and destruction of the resin matrix, which result in further loss of the matrix compared with the inorganic phase. Some other researchers believe that the matrix of composite resins is more resistant to mechanical and chemical challenges and attribute the surface hardness to hydrolytic changes in water, predominantly at the filler matrix interface.^{25,30} Low concentrations of hydrogen peroxide have no significant effect on the surface hardness of composite resins; however, even concentrations higher than those recommended by the manufacturer have no deleterious effects on composite resin surfaces.²⁵

El-Murr et al,²² too, showed that SR of composite resins increases significantly after bleaching, but since it was $<0.2\text{ }\mu$, it was considered clinically important. Attin showed that storage in saliva might decrease the deleterious effects of bleaching by forming a superficial salivary protein layer on the restorative materials.³⁶ Mortazavi et al²⁰ showed that SR of microhybrid composite resins does not change after bleaching, which was attributed to the size of fillers in these composite resins that prevent changes in SR even when resin is lost. In the present study, too, bleaching did not increase the SR of giomer and microhybrid composite resin, consistent with the



results of studies by Mortazavi et al,²⁰ Moraes et al,³² and Polydorou et al.³³ Therefore, the second hypothesis was confirmed.

The results of the present study showed that both the type of the restorative material and bleaching significantly affected BA, with greater adhesion in microhybrid composite resin without any bleaching procedure.

Many factors affect biofilm formation, including SR, free surface energy, and the chemical composition of the surface.³⁷ In relation to the surface chemistry, it might be pointed out that release of fluoride from giomer^{13,16,27,38,39} might result in a decrease in microbial adhesion. Some studies have shown that BA of giomer is less than that of other composite resins, which is attributed to the chemical structure of giomer.^{16,27} In fact, giomer has an antibacterial effect, which is related to its fluoride release capacity.^{13,16,27,38,39} It has been shown that specific monomers are released from composite resin that promote the growth of cariogenic bacteria.⁷ Eick et al,⁴⁰ too, showed higher or similar BA of composite resins compared with ceramics and attributed it to the presence of specific monomers or fillers in composite resins that promote BA. In relation to free surface energy, it has been shown that the majority of metallic alloys have antibacterial properties; however, composite resin samples are different from each other.⁴¹ These studies are consistent with the present study, in which microhybrid composite resin exhibited more BA and giomer exhibited less microbial adhesion.

There is some controversy over the effect of bleaching on BA. The results of the present study showed that bleaching decreased BA. Bleaching might also affect the chemical composition of the surface enamel and restorative materials, changing the adhesion process.²⁹ Bleaching might result in a decrease in the adhesion of salivary proteins on the surface of bleached materials through factors containing peroxide, and it appears it might affect the adhesion of cariogenic bacteria, such as *S. mutans* and *S. sobrinus*.³⁶ Gurgan et al⁴² reported an antibacterial effect for 10% carbamide peroxide, consistent with the results of the present study. However, in a study by Montanaro et al,⁵ none of the bleaching agents was able to decrease the number of the microorganisms tested. Some other studies have shown an increase in BA to bleached enamel.^{17,19,42} Mor et al⁴ showed that *S. mutans* exhibited a stronger adhesion to bleached composite resin surfaces, reporting that bleaching results in some microsurface characteristics on hard surfaces in the oral cavity, paving the way for adhesion of *S. mutans*. Sucrose was used in that study, which has an important role in the adhesion of *S. mutans*. In the present study, saliva was used, which has a protective role for bleached surfaces.³¹ Therefore, it might be pointed out that in the present study, the lower

rate of adhesion to giomer surfaces was due to the release of fluoride from giomer and its antibacterial effect. In relation to bleaching, too, lower adhesion might be attributed to the antibacterial properties of 10% carbamide peroxide, use of artificial saliva, and no use of sucrose. Therefore, the third hypothesis was confirmed.

In the present study, based on Spearman's correlation coefficient, there was no correlation between BA and SR. In the previous studies, surface properties have been reported to be an important factor for BA.¹⁴ In relation to *S. mutans*, which has the strongest relationship with recurrent caries,⁶ adhesion is first initiated by electrostatic bonds, followed by the activation of extracellular enzymes, such as glycosyl transferase.²⁹ Studies have shown that the initial colonization of bacteria begins at surface irregularities, where the bacteria are protected against sheering forces. It has also been demonstrated that restorations with SR promote adhesion of glucans and bacterial colonization.³⁰ Yamauchi et al¹⁴ showed that the effect of SR on BA depends on the type of the bacterial species. For example, *S. mutans* exhibited better adhesion to smooth surfaces; however, *Porphyromonas gingivalis* exhibits better adhesion to rough surfaces. Mei et al⁴³ showed that *S. mutans* adheres to rough surfaces less tenaciously and adhesion of *S. mutans* to composite resin is affected by SR to a lower degree compared with *Streptococcus sanguis*.

Some studies have shown an increase in BA with an increase in SR.^{16,18,19,27} Some other studies have shown no relationship between SR and BA.^{17,18,41} Rosentritt et al⁴¹ showed no correlation between SR and BA, reporting an SR value of <0.08 μ in all the samples. The acceptable threshold of SR is believed to be 0.2 μ and if restorative materials have SR higher than the threshold, there will be an increased risk of plaque accumulation, gingival inflammation, and dental caries.^{30,44} Yamamoto et al¹⁵ showed no relationship between SR and BA. Since restorative materials are polished very well, differences in BA are due to the properties and chemical composition of materials, consistent with the results of the present study. Therefore, the fourth hypothesis was refuted.

The results of the present study showed that since the currently available composite resins have high polishability, there will be no major changes in the SR after bleaching. In addition, since a threshold of 0.2 μ should be considered for SR for BA, it might be concluded that SR will have no effect on BA. However, further studies are recommended to evaluate the following: (1) The relationship between hydrophilic changes in giomer and its SR; (2) The effect of bleaching on BA in the oral cavity.

CONCLUSION

It was concluded based on the results of the present study as follows:

- The type of the restorative material had a significant effect on SR and Ra was higher in giomer; however, bleaching had no effect on SR
- Both the types of the restorative materials and bleaching procedure had significant effects on BA. Bleaching decreased adhesion of *S. mutans* to both materials. Giomer exhibited less BA compared with microhybrid composite resin. In general, microhybrid composite resins without bleaching exhibited greater BA
- Based on the results of Pearson's correlation coefficient, there was no correlation between BA and SR.

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